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Coffee berry disease in Kenya. I. Colletotrichum spp. colonizing the bark of Coffea arabica

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Abstract

Several species of *Colletotrichum* occur in maturing bark of *Coffea arabica* branches in Kenya. The *Colletotrichum* population inhabits the bark tissue external to the developing phellogens in the cortex. The *Colletotrichum* species are unable to invade green bark tissue, where the phellogen has not yet been differentiated, while colonization ceases on the phelloderm of the true bark.

Only one of the *Colletotrichum* species discussed in this paper, *C. coffeanum*, can infect green coffee berries. Shortly after the initiation of the first phellogen in the cortex the parasite is in a small area in the bark. It cannot be found in bark tissues where more phellogens have been formed and where the colour of the bark has changed from yellow-green to brown or black.

From the bark of *C. liberica* trees, grown in Kenya, and *C. arabica* cv. 'Bourbon', grown in greenhouses in the Netherlands one of the saprophytic components of the *Colletotrichum* population could be isolated.

It was impossible to induce die-back symptoms or mere infection by inoculation of green internodes even after wounding of live branches of *C. arabica* with any of the *Colletotrichum* components colonizing the bark. It is suggested that die-back systems of coffee in Kenya are primarily caused by unfavourable growing conditions.

Introduction

Colletotrichum coffeanum Noack occurs on *Coffea arabica* L. sometimes as a pathogen but mainly as a saprophyte in all coffee-growing areas of the world (Noack, 1901; Butler, 1918; Small, 1926; Gutierrez, 1954 and Meiffren, 1957). Recently Saccas and Charpentier (1969) reported the occurrence of *C. coffeanum* on *C. canephora* Pierre ex Froehner (Robusta coffee) and *C. dewevrei* d. W. & Dur. (Excelsa coffee) in the Central African Republic.

In 1922 the first definite report of a fungal attack on green coffee berries appeared on the files of the Department of Agriculture in Kenya (Rayner, 1952). Macdonald (1926) described the causal agent as a pathogenic form of *C. coffeanum*. The losses due to berry infection and berry drop were serious and the disease was subsequently named coffee berry disease (CBD).

With CBD gradually spreading through all high-altitude coffee-growing areas (above 1800 m.) of Kenya, research efforts were concentrated on the control of the disease (Macdonald, 1937; Rayner, 1952). These efforts, however, met with little success. Around 1951 CBD had established itself in all high-altitude areas in Kenya. A separate CBD research-unit was founded in 1955 to investigate all aspects of the disease.

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As a result of the work of the CBD research-unit, Nutman and Roberts (1960 and 1961) reported that *C. coffeanum* habitually colonizes the maturing bark external to the developing phellogens in the cortex. They suggested (1961) that the pathogenic form, which probably arose as a mutant before 1922, had displaced the saprophytic form of *C. coffeanum* in the cortical tissues of bearing wood in heavily affected CBD areas. These workers showed that the sporulation of the pathogen on the bearing wood supplied inoculum for both flowers and fruits, while under certain weather conditions the pathogen in the bark would be able to induce 'Elgon die-back' symptoms (Nutman and Roberts, 1960). Reduction of this 'inoculum potential' (Nutman and Roberts 1961) by applying fungicidal sprays before the onset of the rains would give a better and more economic control, than protecting developing berries. Bock (1963) reported favourable results with pre-rain copper sprays.

Shortly after the CBD research-unit had been dissolved and after the very wet 1961–1962 period losses due to the disease increased alarmingly when the low- altitude coffee areas (below 1800 m), previously free of the disease, became infested with CBD. New research efforts were initiated in 1964. At that time it became apparent that the recommended spray programme of pre-rain copper applications was partially or totally ineffective (Wallis and Firman, 1965). A reassessment of earlier work was considered necessary, also because of information obtained from other African countries where CBD occurs (Muller, 1964; Mendes da Ponte, 1966; Butt and Butters, 1966).

Recently Gibbs (1969) and Hindorf (1970) reported in detail on the various components of the *Colletotrichum* population, isolated from *C. arabica* in Kenya. In view of the confused state of the taxonomy of *Colletotrichum* the former author used the generic name alone for the four groups of isolates by him on coffee branches. Hindorf made the following classification for his isolates, based on their cultural characteristics:

1. *C. coffeanum* Noack (Rayner, 1952: *C. coffeanum* var. *virulans*; Gibbs, 1969; CBD),
2. *C. acutatum* Simmonds (Gibbs, 1969: *ccp*),
3. *C. gloeosporioides* Penzy (Gibbs, 1969: *ccm*),
4. *C. gloeosporioides* (Vermeulen, 1970),
5. *C. gloeosporioides* (Gibbs, 1969: *cca*) and
6. *Glomerella cingulata* (Stonem.) Spauld. & v. Schr.

In this classification isolate 1 is the causal agent of the coffee berry disease while isolates 2, 3, 5 and 6 are saprophytes. The mildly parasitic nature of isolate 4 will be discussed at a later stage (Vermeulen, 1970).

This paper will cover the anatomical aspects of bark maturation in relation to the colonization of cortical tissues by the various isolates of *Colletotrichum* (Rayner, 1948; Mulder and Hocking, 1967) and the sporulating capacity of bearing wood of coffee branches (Nutman and Roberts, 1961). The classification given by Hindorf (1970) will be used in this paper.

Materials and methods

To investigate the invasion of the fungus in the cortical tissues in relation to bark maturation (Nutman and Roberts, 1961) branches of three coffee varieties ('Harar', 'S.L. 34' 'Mocha'), susceptible to *C. coffeanum*, were cut off from trees at the National

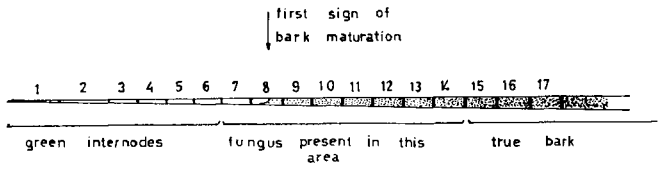


Fig. 1. Longitudinal diagram of a coffee branch. The internodes are numbered from the apex to the basal part of the branch. The position of internodes showing the first signs of bark maturation can differ from one branch to another. *Colletotrichum* spp. are only present in internodes 7–14, not in green internodes or in true (fully mature) bark.

Fig. 1. Lengtediagram van een koffietak. De internodiën zijn genummerd vanaf de top naar het oudere gedeelte van de tak. De plaats van internodiën met het eerste teken van bastrijpheid kan van tak tot tak verschillend zijn. Colletotrichum soorten zijn alleen te vinden in internodiën 7–14 en niet in de groene internodiën of in de internodiën waar de bastrijpheid volkomen is.

Agriculture Laboratories, Nairobi, Kenya, altitude 1700 m. Three or four branches per variety were divided into internodes and placed serially into screw-top jars, each with a number indicating the position of the specific internodes on the branch and the degree of bark maturation (Fig.1), The internodes in the bottles were washed for three minutes in 0.1 % $HgCl_2$ and rinsed twice with sterile water.

Pieces of about 1 mm^2 were cut aseptically from the bark of successive internodes and plated out on water or malt agar. From each internode a series of at least seven or eight pieces of bark was taken along its length, progressing from the distal part towards the basal part of the internode. Any fungus growth originating from the bark pieces on the agar plates was transferred to malt agar after three to four days. The cultures were identified after two to three weeks The conidia of *C. coffeanum*-like cultures were used for inoculation on green berries in order to test each isolate for pathogenicity (Bock, 1956).

Material treated as described above was also taken from *C. liberica* Bull. ex Hiern. and *C. arabica* cv. 'Bourbon' trees. The former trees were grown at the National Agriculture Laboratories, Nairobi, Kenya and the latter in a greenhouse of the Department of Tropical Crop Husbandry of the Agricultural University, Wageningen, The Netherlands.

Inoculation experiments were carried out with the *Colletotrichum* isolates 1, 2, 3 and 5 obtained from maturing bark to assess their capability to invade green internodes of live branches of Arabica trees (cv. 'S.L. 34') and induce symptoms similar to die-back symptoms (Thorold, 1945; Nutman and Roberts, 1960). Agar discs from pure cultures of each of the strains were placed on internodes, which had first been surface sterilized with 0.1 % $HgCl_2$ and washed with water. The inoculum was kept moist with wet cotton-wool and fixed with a piece of polythene sheet around the internodes. After applying the inoculum around the internodes, half of the treated internodes was wounded by needle-pricks.

Of all internodes freehand, freeze microtome and paraffin cross sections were made to study the anatomical changes in the cortex of the maturing bark in the course of the invasion by the components of the *Colletotrichum* population. For the freehand and freeze microtome sections cotton blue-lactophenol or Sudan IV stains were used. The paraffin sections were stained with thionin-light green – orange G, according to Margolena's method (Gray, 1954, p.499).

Results

Nine extensive sets of experiments were carried out from March 1969 to June 1969. In all experiments attention was paid to the colour of the pieces cut from the bark material. It was found that the bark maturation progress often differed from one branch to another on the same tree. Sometimes, also, internodes were already yellow-brown or brown on top, while they were still green on the lower side of the same internode. Therefore, it was decided to use the colour of the bark as a criterion, rather than the actual position of the internode on the branch (Fig.1). In Fig.2 the pieces of bark taken from the internodes are grouped according to colour of the bark. From each group 200 pieces of bark were taken, giving a total of 1200 pieces of bark plated out. It can be seen that out of the six *Colletotrichum* isolates defined by Hindorf (1970,) isolate 3 was found most frequently in the groups green II, yellow, yellow/brown I and brown II, followed by isolate 2 and 5. *C. coffeanum*, the causal agent of CBD, could be found exclusively in groups green II, yellow and yellow/brown, though it occurred even there at low frequency, In the cortex of these tissues the first phellogen had just been initiated (Fig. 3) near the stonecell-layer, and the exterior cortical tissue had been cut off very recently from the cambial tissue by the phellogen. As soon as more phellogens had been formed (Fig. 4) and the colour of the bark had changed from yellow-green to brown no *C. coffeanum* isolates could be obtained from the bark tissue. No growth of any *Colletotrichum* has been detected in green bark tissues which did not show signs of phellogen formation (Fig. 5). On the true bark, where two or three phellogens had been formed, no *Colletotrichum* isolates could be obtained either.

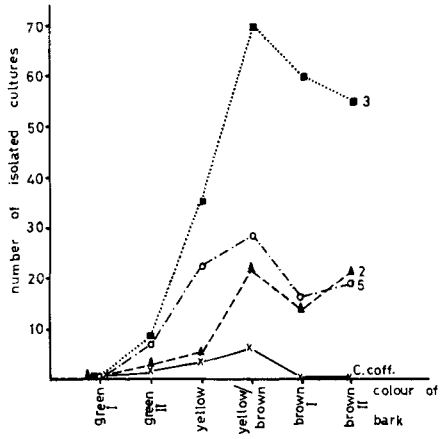
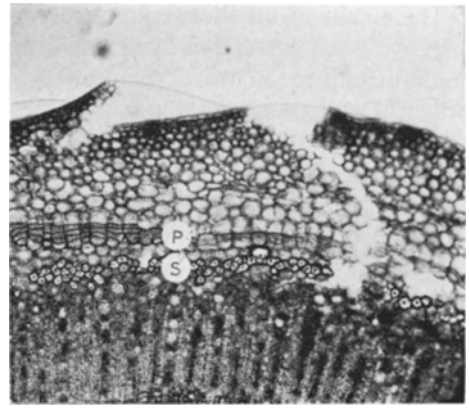


Fig. 2. Total of all cultures of four *Colletotrichum* isolates obtained in nine tests from 1200 pieces of bark cut from the area where *Colletotrichum* is present and plated out on agar. These pieces of bark material are not grouped according to the actual internode position, but according to the colour of the bark; from each group 200 pieces of bark were taken:
green I: completely green bark tissue, no phellogen in cortex;
green II (bordering yellow bark); yellow and yellow/brown: one phellogen in cortex; brown I (bordering yellow/brown bark) and brown II: brown bark tissue, two phellogens in cortex.
C. coff. = *C. coffeanum* = CBD; 2 = *C. acutatum*; 3 = *C. gloeosporioides*; 5 = *C. gloeosporioides.*

Fig. 2. Totaal van alle cultures van vier Colletotrichum isolaten verkregen in negen proeven uit 1200 stukjes bast van het gebied waar Colletotrichum aanwezig is en uitgelegd op agarvoedingsbodem. Deze stukjes bast zijn niet ingedeeld volgens de positie van de internode op de tak, maar ingedeeld volgens de kleur van de bast; van iedere groep waren 200 stukjes bast genomen: groen I: volkomen groen bastweefsel, geen fellogeen in de cortex; groen II (liggend naast de gele bast), geel en geel/bruin: één fellogeen in de cortex; bruin I (liggend naast geel/bruine bast) en bruin II: bruin bastweefsel, twee fellogeenlagen in de cortex.
C. coff. = C. coffeanum = CBD; 2 = C. acutatum; 3 = C. gloeosporioides; 5 = C. gloeosporioides.

Fig. 3. Cross section through green-yellow bark of a coffee branch. The first phellogen (P) has been formed just outside the stonecell layer (S). Magnification about × 130.

Fig. 3. Dwarsdoorsnede van groen-gele bast van een koffietak. Het eerste fellogeen (P) is gevormd juist buiten de steencellaag (S). Vergroting ongeveer 130 ×.



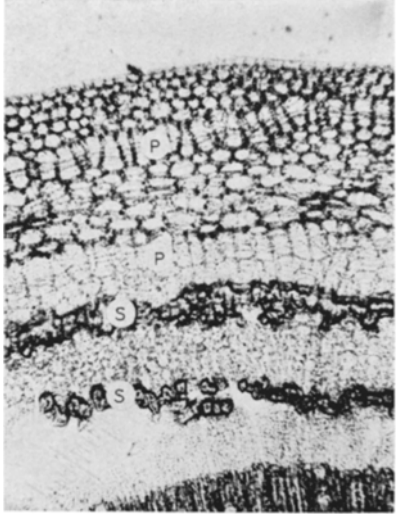


Fig. 4. Cross section through brown bark of a coffee branch. Two phellogens (P) and two stone-cell layers (S) are now present in the bark. Magnification about × 130.

Fig. 4. Dwarsdoorsnede van bruine bast van een koffietak. Twee fellogeenlagen (P) en twee steencellagen (S) zijn nu aanwezig in de bast. Vergroting ongeveer 130 ×.

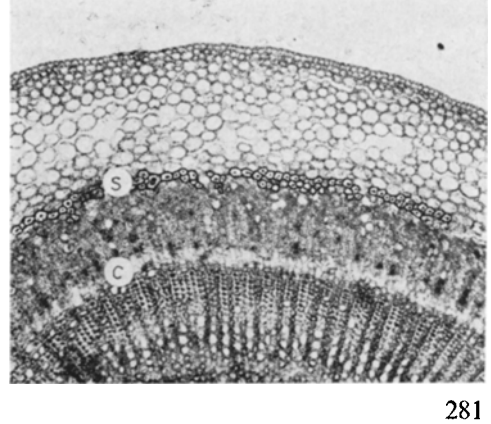


Fig. 5. Cross section through green bark of coffee branch. Outside the cambial tissue (C) only one stonecell layer (S) has been formed. Magnification about × 130.

Fig. 5. Dwarsdoorsnede van groene bast van een koffietak. Buiten de cambiale laag (C) is slechts één steencellaag gevormd. Vergroting ongeveer 130 ×.

The results of the inoculation experiments with *Colletotrichum* isolates 1, 2, 3 and 5 on live green internodes always yielded negative results and no symptoms of die-back could be induced in any of the trials even after wounding the green tissue with needle pricks. This agrees with the conclusion from the results mentioned above, that the *Colletotrichum* isolates are not able to invade tissue where no phellogen has been formed.

The isolates made from *C. liberica* and *C. arabica* cv. 'Bourbon' yielded in all cases isolate 3. The 'Bourbon' material had been maintained in greenhouses for at least twelve years and it seems likely that the fungus was already present in the coffee cuttings imported into the Netherlands.

Discussion

From the data presented in this paper it becomes evident that *C. coffeanum* could only be isolated infrequently from green II, yellow and yellow-brown bark tissues (Fig. 2), where the first phellogen had been formed. The other, saprophytic isolates are present more frequently in the same tissues (respectively approximately ten and twenty times higher than *C. coffeanum*), and continue to be present in the brown bark (Fig. 2, brown I and II respectively). This means that on a bearing branch the role of *C. coffeanum* in the *Colletotrichum* population is usually relatively unimportant and the presence of the parasite is virtually restricted to a small area just apical of the zone with brown colouration. The observations of Nicholls (1969) indicate, however, that there can be appreciable increase in the amount of yellow bark in the early part of the Long Rains (March-April) on certain types of branches. Considering the data presented in this paper the increase of yellow bark surface would mean a higher incidence of *C. coffeanum* and this might explain the CBD-outbreaks during or shortly after the rains. Data presented by Gibbs (1969) and Hindorf (1970), similarly show the relatively low proportion of *C. coffeanum* in the *Colletotrichum* population. The fact that *C. coffeanum* cannot occupy brown cortical tissue has most likely nutritional reasons. No explanation can as yet be offered for the fact that the saprophytic isolates of *Colletotrichum* are also capable of occupying green II and yellow bark. The hypothesis put forward by Mulder and Hocking (1967), that the saprophytes and the parasite would occupy cell-layers at different depths in the cortical tissues might apply to the green-yellow tissues.

Furthermore it becomes clear that the conidia produced on the eight internodes specified by Nutman and Roberts (1961) will be mainly conidia of the saprophytic isolates. Vermeulen (1968) proposed the term 'potential conidial production', covering all *Colletotrichum* conidia produced on the branch. More recently the term 'sporulating capacity' (s.c.) has been introduced (Gibbs, 1969; Griffiths and Gibbs, 1969; Nutman and Roberts, 1969). With our present knowledge of the ratio saprophytic-parasitic conidia produced on the bark it is easy to understand why Wallis and Firman (1965) and Vermeulen (1968) had difficulties to relate their s.c. findings with actual disease incidence.

It should be kept in mind that the data presented in this paper apply to an altitude of approximately 1700 m. Hindorf (personal communication) has observed variations in the composition of the *Colletotrichum* population with the altitude. The role of *C. coffeanum* in the whole population remains, however, relatively small at all altitudes.

The failure to induce die-back symptoms in green internodes of healthy coffee branches with the *Colletotrichum* isolates 1, 2, 3 and 5, even after wounding the bark tissues, suggests that die-back symptoms of branches are indeed most likely to be caused primarily by unfavourable growing conditions (Thorold, 1945). Under adverse conditions the ubiquitous *Colletotrichum* may invade the green bark tissues and even attack the cambial layer, thus causing die-back symptoms. No experimental evidence could be obtained that *C. coffeanum* is able to invade green internodes of live branches (Nutman and Roberts, 1960).

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Samenvatting

Koffiebesziekte in Kenia. I. Colletotrichum spp. die de bast van Coffea arabica koloniseren.

Een aantal soorten van *Colletotrichum* komt voor in de rijpende bast van *Coffea arabica* takken (Fig. 1 en 2) in Kenya. De schimmelpopulatie koloniseert het bastweefsel, dat zich in de cortex buiten de ontwikkelende fellogeenlagen (Fig. 3 en 4) bevindt. Deze schimmels zijn echter niet in staat groen bastweefsel te koloniseren, waar nog geen fellogeen (Fig. 5) is gevormd. In het deel van de tak waar echte schors voorkomt, met in de cortex twee tot drie fellogeenlagen, kon geen *Colletotrichum* worden geïsoleerd. De *Colletotrichum* soort, die groene koffiebessen kan infecteren, *C. coffeanum*, is slechts sporadisch te vinden in een klein gebied van de bast kort na de vorming van het eerste fellogeen (Fig. 3) in de cortex. In bastweefsel waar meer fellogeenlagen zijn aangelegd en de kleur van de bast van groen-geel tot bruin of zwart is veranderd, komt *C. coffeanum* niet meer voor, maar wel andere *Colletotrichum* soorten (Fig. 4).

Met geen van de *Colletotrichum* soorten – beschreven in dit artikel – konden instervings-symptomen worden geïnduceerd na kunstmatige infectie van groene internodiën op levende takken. Het was zelfs niet mogelijk na verwonding van de bast infectie te verkrijgen van het bastweefsel, waarin nog geen fellogeen was gevormd. Het lijkt daarom waarschijnlijk dat ongunstige groeiomstandigheden de primaire oorzaak zijn van instervings-symptomen, waargenomen bij koffie in Kenya.

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